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SARCOSPORIDIA ENCOUNTERED IN PANAMA

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From Board of Health Laboratories, Ancon, Canal Zone.

During the routine examination of nearly one thousand animals that have come to autopsy here and have been specially examined for parasites, Sarcosporidia have been detected in three new hosts: opossum,[1] hawk and sloth. The following animals were also found to be parasitized: horse, cow, hog, sheep, cat, man[2] and rat, *Mus. rattus* and *Mus. norvegicus.*[2]

Most of the sarcocysts of the common species were found to resemble closely those described from these animals by other writers. The sarcocyst of man, however, presented a different appearance from the one described by Baraban and St. Remy from the laryngeal muscles of man in that the former exhibited younger and apparently abortive cysts. Sarcosporidiosis appears to be far more widespread among various animals than has been supposed. Some host species appear to show a constantly high incidence of infection; others, as man, for example, seem rarely infected.

The infection is a common one, but it probably has little pathological significance or economic importance. It is extremely rare here to find an animal other than a rat visibly infected and those sarcocysts encountered microscopically display little or no evidence of having induced any tissue reaction.

Some animals retain permanently evidences of the infection, but my observations in a case of infection[2] of a negro lead me to believe that sarcocysts may be abortive and after a short residence in the muscle fibers disappear in various ways.

Interest in Sarcosporidia centers in their life cycle, and considering the large number of infected animals accessible for experimentation, it is remarkable that so little light has been thrown on the subject. This is very largely due, no doubt, to the view that the sarcocysts are not pathogenic[3] and are of doubtful economic importance.

Sarcosporidia of birds have been described by Kühn, Leidy, Rivolta, Barrows and Stiles,[4] the following birds having been parasitized: common fowl, *Gallus,* blackbird, raven, shoveler, spoonbill duck, mallard duck, grosbeak, and two Brazilian birds, *Aramadis saracura* and *Ammodromus manimbe.*[5]

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[1] Bull. de la Soc. Path. Exot., iii, p. 513, 1910.

[2] Arch. Int. Med., April, 1909.

[3] McGowan: Investigation into the disease of sheep called "Scrapie." Blackwood, Edinburgh, 1914, p. 116, believes that "Scrapie" is due to Sarcosporidiasis.

[4] See Bull. No. 3, B. A. I., U. S. Dept. of Agr., 1893.

SARCOCYST OF THE HAWK: *Leucopternis* SP.

The sarcocysts were detected microscopically and had invaded the striated muscles of the leg, and presumably elsewhere. The muscle fibers of smaller size were usually invaded, for the sarcocysts in cross section were 29 and 20 μ in diameter, while the diameter of the muscle fibers nearby was only 16 μ.

Muscle fibers of larger size were also parasitized for one sarcocyst was 48 μ in diameter with a limiting membrane or false capsule of muscle substance 6 μ in thickness. This was not striated and was of lighter color than neighboring muscle fibers which were 32 μ to 44 μ in diameter (Figs. 3 and 4).

The sporozoites were 4.5 μ long and 1.2 to 1.5 μ wide with a nucleus in one end apparently about 1.0 μ in diameter. The sporozoite stains rather diffusedly with hematoxylin. A definite reticulum cannot be made out, but there are clear spaces within the sarcocyst indicating the presence of sporoblastic chambers.

A few small parasites 12 μ in diameter have invaded the muscle fibers of the heart and here they have a thin but definite limiting capsule and are located in the inner circular layer. None was seen in the outer longitudinal layer of fibers.

SARCOCYST OF THE SLOTH

The tissues of this animal *Choloepus didactylus* as well as of several other individuals were searched for sarcocysts, but not until a microscopic examination of sections of muscle was made were any found, the sarcocysts being found in the esophagus and muscles of limbs and trunk.

The sarcocysts were always found within the muscle fiber enclosed in a very thin capsule. One sarcocyst measured 0.033 mm. in diameter, its muscle fiber being 0.051 mm. in diameter. Another occupied the entire width of the muscle fiber. The muscle of the esophagus was more highly infected than other muscles and the sarcocysts were more frequently found in its middle longitudinal layer of fibers.

None was found in the heart. Not all the sections of muscle examined were parasitized, however, and none was found in viscera examined: kidneys, lymph nodes, spleen, liver and lungs. In the infected muscle the sarcocysts were packed with sporozoites or sporoblasts, which were either banana-shaped or round. Isolated spores were

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[5] See Splendore, Rev. d. Soc. de São Paulo, pp. 115-120, 1907.

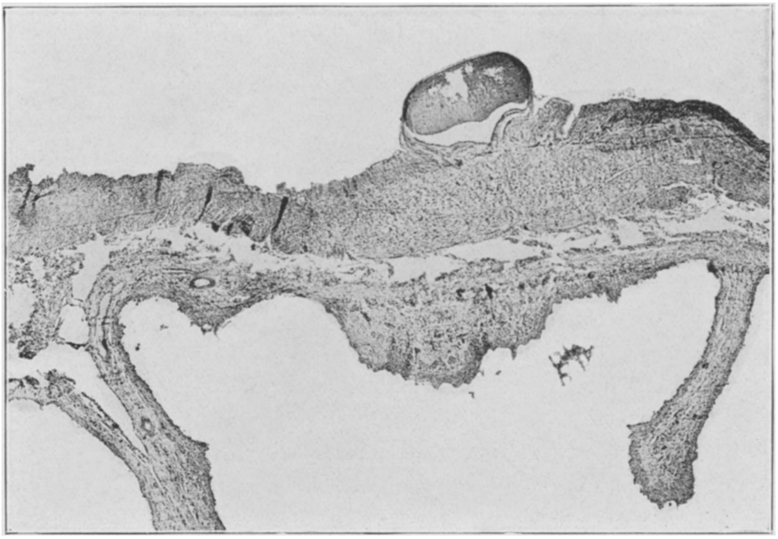

Fig. 1

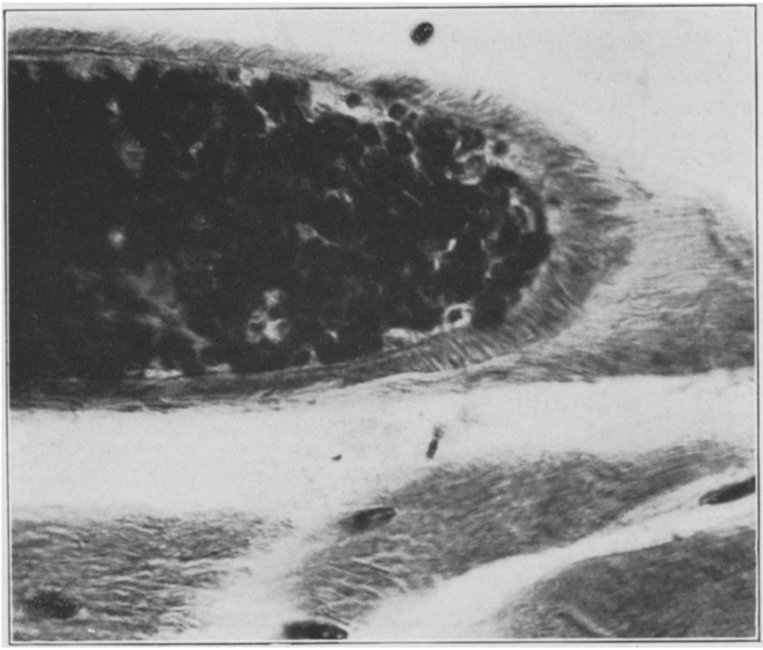

Fig. 2

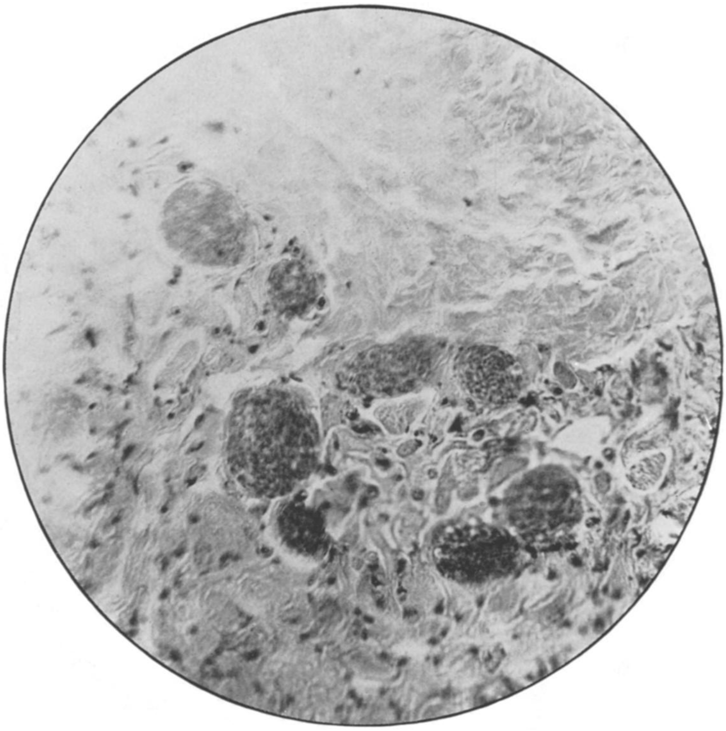

Fig. 3

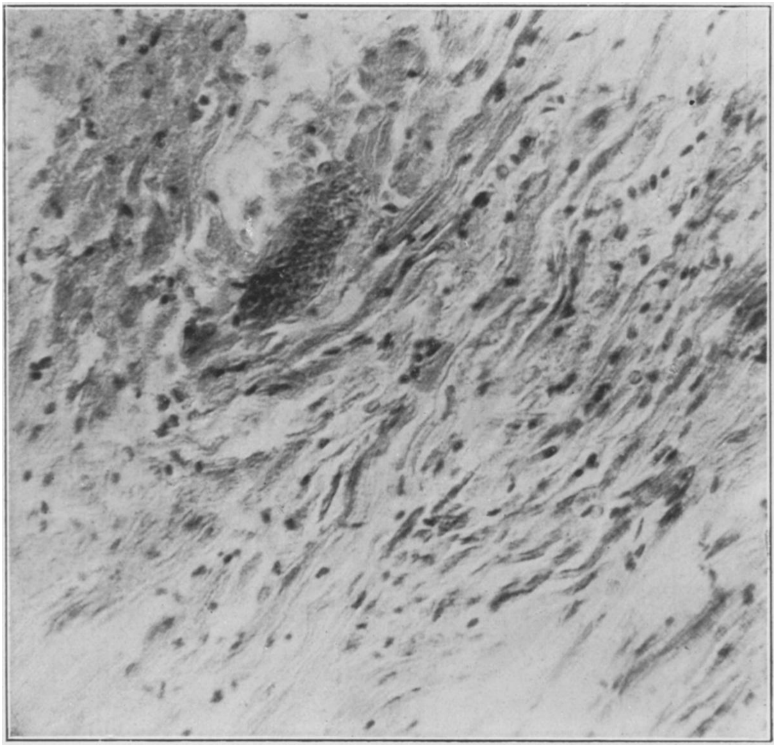

Fig. 4

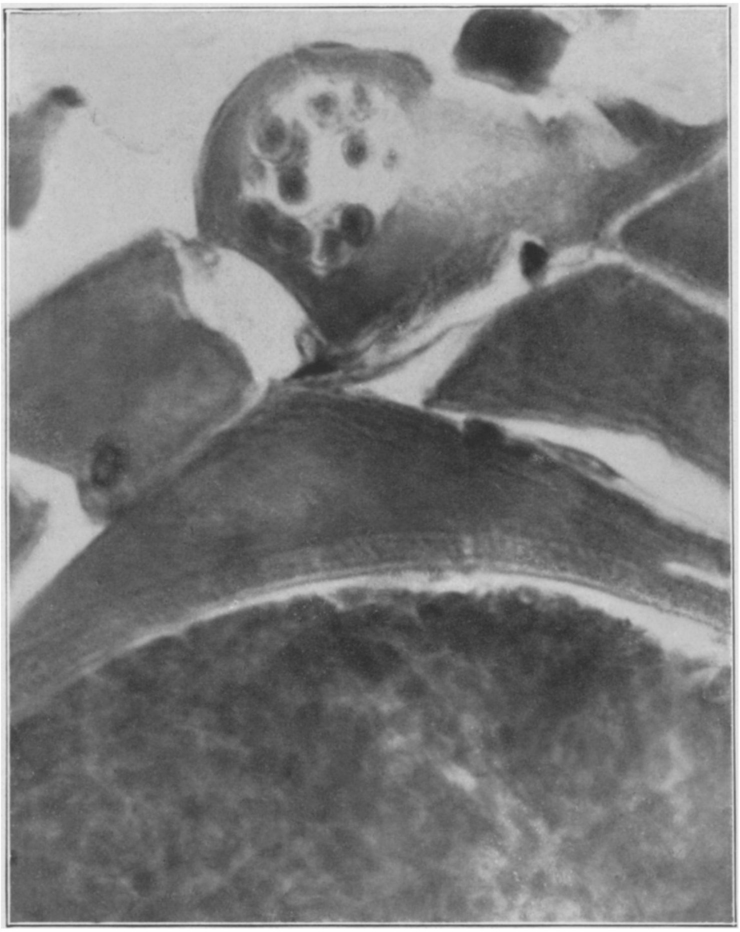

Fig. 5

examined with difficulty on account of their being so closely packed together in the cyst.

Altogether the sarcocysts resembled those seen in man, opossum, and guinea-pig experimentally infected with *S. muris.* Apparently, there are two well-marked types of sarcocysts, those with large sporoblasts seen in rat, sheep, cattle and horse, and the type with very small sporozoites or sporoblasts encountered in man, sloth and opossum.

Considering the results of the experiments of Negri, and the writer, with "*S. muris*" as well as those of Erdmann with "*S. tenella*" in which there was a reduction in the size of the sporozoite encountered in the subinoculated animals it seems likely that the size of the sporozoites in the sarcocyst may depend on the host and be irrespective of the variety of sporozoite inoculated.

SARCOCYST OF THE OPOSSUM

This remarkable parasite was found in the opossum *Didelphis* sp. distributed to the voluntary muscles and heart and also in the lungs, stomach, small intestine (Fig. 1), and its mesentery, pericardium, submaxillary gland, and esophagus, but not in the kidney, liver, spleen, pancreas, bladder, gall-bladder, external genitalia, or eye and extrinsic muscles. For this reason the definition of Sarcosporidia must be amended to include representatives which from the very start lodge and develop in non-muscular tissue.

All the parasites were of one size and age.

The attempt was made to infect guinea-pigs by the intramuscular inoculation of sporozoites; and in one of two guinea-pigs so inoculated with the opossum parasite and sacrificed sixty days after inoculation, Sarcosporidia were found in sections of muscle from the leg inoculated. The other guinea-pig, when sacrificed after a period of 146 days failed to disclose parasites in tissue from either leg.

The Sarcosporidium found is believed to be of an abortive type and probably does not experience further development, but dies out early. The feeding habits of the host suggested at the time that the parasite might have been derived from poultry, and one out of a good many fowl examined, a black Orpington, contained lime-infiltrated bodies grossly not unlike the Sarcosporidia found in the opossum. While the rat, cat, hawk and other animals revealed Sarcocysts of different ages, in the opossum they were all of one age. Apparently there had been but one infecting period and no auto-infection as described by Negri in other forms.

SARCOSPORIDIA OF THE CAT

Wasielewski[6] states that Sarcosporidia have been found in the domestic cat, and Railliet[7] quotes Krause as having discovered these parasites in the ocular muscles of the cat.

A vagrant cat, much emaciated and dying of starvation, was caught near the laboratory. After being chloroformed it was noted that a large chicken bone had become wedged between the back teeth effectually preventing the use of the jaw. There was found a severe itch mite dermatitis of both ears and a number of intestinal cestodes and nematodes.

No gross evidences of sarcosporidiosis were noted at autopsy, but from the size of some of the parasites detected later in sections of tissue the infection must have been visible macroscopically if the muscle had been more carefully scrutinized.

Microscopically, there was extreme atrophy of the spleen and vacuolar degeneration of renal epithelium as in starvation. Striated muscle contained many parasites 24 to 160 μ in diameter, the normal muscle fibers nearby being 24 to 40 μ in diameter. There was an extremely delicate limiting membrane with delicate prolongations suggestive of sporoblastic chambers. The muscle substance enclosing the parasite was paler in color and more refractile and granular than neighboring muscle. In the larger parasite the sporozoites or sporoblasts were in agglomerated masses.

A few very young parasites were seen containing sporoblasts (Fig. 5) very much like those of *S. tenella* and there was no limiting membrane or capsule visible. The sporozoite in sections appears to be about 7.5 μ by 1.5 μ.

SARCOCYST OF MAN

This was detected during life in the biceps of a young negro.[2] Examination disclosed small sarcocysts one-third to one-half the diameter of the muscle fiber, distended with vesicular sporoblasts. The parasite was of the minute or abortive type such as Negri and I have found in guinea-pigs fed with sarcocysts from the rat and differed from those described from the laryngeal muscle of a man by Baraban and St. Remy in that the latter were much larger, possessed sporoblastic chambers, and were of a more mature type. Four months after the parasite had been detected, another examination of tissue showed that apparently they had entirely disappeared.

SARCOCYSTIS MURIS

This form was frequently encountered during the routine bacteriological examination of rats from Panama and the Canal Zone. The young and immature rats usually showed no signs of gross infection, but when muscle was examined microscopically small sarcocysts were sometimes detected. Out of 131 adult rats examined grossly, 15 or 11.4 per cent. were infected. *Mus norvegicus* showed a higher percentage of infection (10.7 per cent.) than *M. rattus* (3.5 per cent.). There was never the slightest indication of cellular proliferation or necrosis in the muscle fiber of the infected rats and the animals all appeared to be in good physical condition.

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[6] Wasielewski, Sporozoenkunde, Jena, 1896, p. 119.

[7] Railliet, Zool. Médic. et Afric., 1895, p. 154.

Feeding experiments were carried out with *S. muris* on guinea-pigs. Four pigs proved negative upon microscopic examination of several blocks of muscular tissue, but after a period of 164 days Sarcosporidia were detected in sections of muscle from two guinea-pigs; the morphology, however, was different from that of the sporozoites fed from the rats in that the former were much smaller and resembled those seen in tissue from the biceps of the human case and in guinea-pigs inoculated intramuscularly with sporozoites from the sarcocysts of an opossum.

SARCOCYSTIS TENELLA

There are no sheep in Panama. Occasionally a lamb is brought in from Peru to be kept as a pet, but for some reason they do very poorly. One such lamb died and was examined at the laboratory. At the autopsy there was a slight amount of pericardial clear fluid, general glandular enlargement and splenization of the lungs. Microscopically, there were many epithelial necroses in the kidneys and a few small Sarcosporidia in muscle fibers of the esophagus 22.5 μ in diameter. These were very young parasite and corresponded to the description of *S. tenella* (see Railliet).

SARCOCYSTIS MIESCHERIANA

During a histological examination of hogs that had died of swine fever, sarcocysts were detected in striated muscle. The hogs were red Jersey, Berkshires and natives, and were bred in Panama. They contained young parasites with very large sporoblasts 3 by 8 μ or larger in sections.

SARCOCYSTIS FUSIFORMIS

Case 1. Material was obtained from an emaciated cow of the hospital dairy herd originally from the U. S. A., that had died of puerperal pyemia. Some large sarcocysts were seen in striated muscle. The muscle fiber enclosing the parasite was altered into two layers, an inner dark or granular layer and an outer paler layer, paler than the muscle nearby (Fig. 2). The sporozoites are very large and apparently packed in room systems.

Case 2. Emaciated cow, from the Ancon dairy herd, originally from the U. S. A. The autopsy findings were bronchial strongyles, liver necrosis and aneurysm of hepatic artery. The esophagus contains some large sized sarcocysts with a definite thin inner lining and a thicker grayish eosin colored capsule with radiating striations (see figure as in *S. tenella*). There is a delicate eosin staining reticulum making the room systems (sporoblastic chamber or capsule).

This sarcosporidian has also been detected in muscle tissue of beef from Argentina.

SARCOCYSTIS BERTRAMI

An emaciated mule from the U. S. A., 14 years old, died suddenly without assignable cause of death. Sarcocysts with large sporozoites 3 by 9 μ with eosin staining cytoplasm were detected in striated muscle.

DISCUSSION

The deepest mystery surrounds the life history of the Sarcosporidia. Minchin says that in the present state of our knowledge the Sarcosporidia would appear to be true Cnidosporidia with spores which contain each a single polar capsule and from which an amebula is liberated as in other Neosporidia.

Theobald Smith[8] succeeded in infecting mice by feeding muscle tissue of infected mice to non-infected animals, and while cannibalism and carnivorous habits may spread the infection among certain animals it is certainly not the way the disease is transmitted to sheep and cattle. Watson, according to Minchin, stated that spores are to be found in the circulating blood, but McGowan believes that Watson was very indefinite on the subject and his remarks may not bear that interpretation.

In attempting to account for the Sarcosporidium of cattle Smith states "their muscle parasite is either an aberrant form from some invertebrate taken in with their food or else there is an intestinal stage . . ." Professor Smith further states that he desists "from any further discussion not based on actual studies."

According to Minchin,[9] who follows Erdmann, the spores germinate in the intestines of the new host. There is a liberation of sarcotoxin which destroys the epithelium. Amebulae are set free which penetrate into lymph spaces of the submucous coat. By analogy this represents the planont or first stage, it is initiated by sexual processes.

The second stage represents invasion of muscle fibers and development of amebulae therein, first by a multiplication of nuclei until about twelve nuclei are seen. The parasite becomes divided up into separate cells or pansporoblasts or sporonts which actively multiply by division. This stage is reached in forty-eight to sixty days. The parasite may disintegrate at this stage setting free sporonts or may develop into a Miescher's tube. If the former, there is wandering out and invading other muscle cells; in the latter development of an enclosing membrane with chambers occurs. Fully formed spores are found in parasites eighty to ninety days after the infection of the host.

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[8] Smith, Jour. of Exp. Med., vi, 1901.

[9] Minchin: An Introduction to the Study of the Protozoa, 1912, p. 422.

Thus, Sarcosporidia belong to a group of sporozoa, the *Neosporidia,* which often occur in invertebrates as in the larva of the silk-worm moth, and the mode of infection is contaminative, i. e., by ingestion of spores on leaves and by an hereditary way in which ova are infected.

Fantham and Porter,[10] in an investigation of the pathogenicity of *Nosema apis* to insects other than hive bees, found that humble bees, mason bees and wasps became infected.

Cabbage plants near a badly diseased hive were found spattered with bee excrement. Dead larvae of cabbage-white butterflies also were found, whereupon it was ascertained that when this larva was fed on spore-infected honey-cabbage the larva became infected and diseased.

Cinnabar and gooseberry moths were infected experimentally. Blowflies, craneflies and sheep keds were also infected experimentally. This investigation of Fantham and Porter's indicates that *Nosema* is capable of causing infection and disease in quite a range of invertebrate hosts and the wideness of the range suggests the possibility that vertebrates might be as easily infected.

The animals most highly infected with Sarcosporidia, i. e., high incidence of infection, are pigs, sheep, cattle and horses, i. e., non-flesh-eating animals. If Sarcosporidia are obligate parasites for mammals and other vertebrates, the contaminative mode of infection from mammalian and vertebrate hosts seems to be out of the question, for the spores cannot be liberated from the muscles, and, so far as we know, do not escape by way of the intestinal tract of the host to infect his environment; nor is it likely that they get into the blood stream.

In the case of mice, rats and carnivorous animals the mode of infection may in part be by way of the intestinal tract through eating infected tissue; but the infection of sheep, cattle and horses cannot be explained in this way.

On account of the facility with which herbivora may obtain and ingest invertebrates infected with Neosporidia, but more particularly flowers, leaves, etc., or water contaminated with droppings of bees, moth larvae, fly droppings, etc., or other material containing Neosporidia spores, is it not possible that Sarcosporidia may be sidetracked varieties of some of the Neosporidia of invertebrates which have invaded the musculature of a hospitable though by no means definitive host and are unable to continue further their life cycle and escape from a compromising and aberrant position? The strikingly high incidence of infection among hogs, cattle, sheep and horses might be explained in this way.

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[10] Ann. Trop. Med. and Paras., vii, 1913, p. 569.

While many parasites are obligate in their host relationships there can be no doubt but that certain protozoa, usually parasitic in invertebrates, may more or less readily succeed in establishing a foot-hold in the tissues of man or other mammal and cause a definite pathological condition therein. Take dermal leishmaniasis for example. In 1910 a solitary cases of this disease was detected here in a man that had been bitten by a tabanid fly out in the bush. Since that date some five or six others have been identified here by the presence of intracellular parasites morphologically identical with *L. tropica*. Now, if *L. tropica* is an obligate specific parasite for man, it is very unlikely that Tabanids or other biting flies are carrying it about in the Canal Zone and only half a dozen cases arise in as many years. At the[11] time I suggested that the sore was the result of an infection by an insect flagellate, and the subsequent incidence of the disease now emphasizes the importance of this view very strongly.

Considering the infrequency of dermal leishmaniasis in this region, the sparseness of the parasites in many of the ulcers and the extreme unlikelihood of an insect being able to penetrate the tough crust of the ulcer or of the papule to regain the flagellate and carry it on, it seems unlikely, assuming that there is a specific form of the virus of oriental sore, that it could be propagated in a region without our being able to detect many more examples of it if it were necessary for its life cycle to be carried out in an insect as well as man.

Under the circumstances it is far more probable that the dermal leishmaniasis of Panama at any rate is "a disease caused by the inoculation of one or more varieties of insect flagellates."

If this should prove to be true then the parasites in the sore are permanently sidetracked in the tissues of man and have no natural way of continuing their life cycle in the variety of insect from which they originally came. It is possible that other protozoa, and among them Neosporidia, meet with similar fates, the latter becoming sidetracked in the muscle fibers as "sarcosporidia" (as well as in other tissues occasionally, such as that of opossum and kangaroo) and while attempting blindly to continue their life cycle in their vertebrate host have no further opportunities for completing the same.

Experiments with *Nosema* or other *Neosporidia* would settle the matter at once, and I regret that no material has been encountered here with which these views on the probable zoologic status of Sarcosporidia might be tested.

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[11] Darling: Arch. Int. Med., Chicago, 1911, vii, p. 581. Proc. Soc. Trop. Med. and Hygiene, Lond., 1910, iv, No. 2, p. 60.